THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

CHEMICAL UTILIZATION OF SOUTHERN PINE BARKS

Project 2753

Report One

A Progress Report

to

MEMBERS OF GROUP PROJECT 2753

February 14, 1969

MEMBERS OF GROUP PROJECT 2753

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SUMMARY

A general analytical processing scheme was developed for the evaluation of the components of southern pine barks. Loblolly and slash pine barks were submitted to sequential extraction with petroleum ether, water, ether, and ethanol. The individual extractives were further fractionated by means of solvent partition, column chromatography, acid hydrolysis, and alkaline hydrolysis, and the individual components analyzed by paper, thin-layer, and gas chromatographies. Major individual moieties present in these barks include stearic acid, oleic acid, linoleic acid, arichidic acid, tetracosanoic acid, hexacosanoic acid, glycerol, octadecanol, tetracosanol, hexacosanol, dihydromyricetin, dihydroquercetin, pyrocatechol, vanillin, vanillic acid, p-hydroxybenzoic acid, oxalic acid, glucose, fructose, galactose, and arabinose. In addition, several unidentified compounds are major components. These include an aromatic acid and several hydrolysis products of waxes. The component make-ups of loblolly and slash pine barks are qualitatively similar and differ only in quantitative composition. The results of this report include large-scale studies of only two species taken in March. The influence of time of year on the yield and nature of components of these two species will be the subject of the next progress report.

INTRODUCTION

For a number of years The Institute of Paper Chemistry has been studying institutionally and for several companies the chemistry of barks of several specific wood species, in most cases with views toward chemical utilization. During this period considerable experience has been gained on the separation of the components of these barks by means of column, paper, thin-layer, and gas chromatographies, ion-exchange, ion-exclusion, and Craig countercurrent distribution and on their identification by means of the qualitative employment of the noted chromatographies standardized with authentic compounds, infrared adsorption spectroscopy, mass spectroscopy, and derivatization. For the obtainment in quantity of compounds both known and unknown to our previous experience, the Institute has been applying the sophisticated procedure of preparative gas chromatography. We have in use a Model 712 Aerograph large-scale automatic preparative gas chromatograph and a Model 770 F & M large-scale automatic preparative gas chromatograph which enable us to collect macroquantities of individual components from complicated mixtures. To aid us in this work, we have perhaps the largest "library" of authentic compounds related to bark and wood chemistry of any laboratory in the world.

More recently, we have been approached by member companies and by allied industry companies to consider the possible chemical utilization of southern pine barks including longleaf pine (Pinus palustris), shortleaf pine (P. echinata), slash pine (P. caribaea), loblolly pine (P. taeda), Virginia pine (P. virginiana), and pond pine (P. rigida). The yield of bark from these species represents 10 to 15% of the total weight of the wood treated, and traditionally, the huge amounts of these barks generated by the wood conversion industries have presented a problem of disposal. In most instances disposal has

been attained by burning — with or without the recovery of heat. Besides creating a fly-ash and air pollution problem, burning appears to be a wasteful manner of treating a potentially valuable chemical raw material, particularly in these times of need for conserving available supplies of raw materials to meet expanding requirements of the chemical industry.

Although considerable success has been obtained in the chemical utilization of some coniferous barks, the almost complete lack of knowledge concerning the components of southern pine barks has precluded similar success in the chemical utilization of these barks. In other words, utilization of this tremendous waste product on a large scale either as a gross product on the basis of its chemical and physicochemical properties or as a raw material for the production of individual chemical compounds must await a fundamental knowledge of the chemical composition of these individual barks.

Such a long-range fundamental research program on the chemistry of the southern pine barks is important to a large segment of the pulp and paper industry and other industries, such as lumbering, chemical, and wood conversion, and is too comprehensive to be undertaken by a single mill or company. On the other hand, it lends itself admirably to group sponsorship. Accordingly, the recent inquiries into possible chemical utilization of southern pine barks, together with the Institute's experience and equipment, our experience in the identification and isolation of wood components, our unusual library of authentic chemicals derived from and related to wood and bark, our continuing interest in the obtainment of valuable by-products from the waste products of the pulp and paper industry, and our anticipation of possible future controls on air pollution, led us to propose this research for a group-sponsored cooperative research project on the chemical utilization of southern pine barks.

The above introductory discussion was submitted in June of 1967 to all member companies in our Proposal No. 1194 for a Group-Sponsored Cooperative Research Project on "Chemical Utilization of Southern Pine Barks" along with the following discussion of a proposed research program.

It is realized that the investigation of the chemical utilization of southern pine barks is an ambitious undertaking because of the variety of southern pines. It is further realized that the particular wood furnish varies considerably from mill to mill, and that the interest of some individual mills may be quite specific. However, the proposed program would be long range in nature and, if supported to the extent desired, would comprise investigations of all southern pine species.

Because Loblolly pine and slash pine are numbers one and two, respectively, in tonnage utilized by the wood conversion industries in the South, we propose to study the barks of these two species first. If sufficient support is obtained for the project, we plan to then study shortleaf pine and longleaf pine, followed by Virginia and pond pines.

In the initial program on this project, we propose to extract the barks with water and with organic solvents. These individual fractions will be determined quantitatively, and the most abundant fractions will be fractionated by the several chromatographic and countercurrent distribution procedures noted in the introductory discussion. These subfractions will be analyzed qualitatively, and the identified components determined quantitatively, all by procedures developed in Institute laboratories and noted in the introductory discussion. Components unknown to our previous experience will be identified. The various types of components expected in these several extracts include flavanoids, carbohydrates, inositols, terpenoids, glycosides, esters, steroids, fats, lignans, complex phenols, tannins, waxes, essential oils, gums, mucilages, resins, etc.

In addition to extractions with water and with several organic solvents, we plan to submit these barks to extraction with boiling sodium hydroxide. Recent experience in this field has indicated that complex moieties in woods and barks are degraded to give monomeric aromatic acids, aldehydes and ketones.

Components which are indicated by the above noted studies to be produced in substantial amount will be studied further. These further studies will include procedures for isolation and purification. Possible market research or commercial development is not contemplated by this proposed program, but may be the subject of further consideration from the several sponsors. The suitability of individual components or fractions for such large-scale uses such as dispersing agents, oil well drilling mud additives, binders, food supplements, etc. will be kept in mind, and utilization possibilities will be suggested to the sponsoring companies.

Although sponsorship for Research Proposal No. 1194 was not obtained in the amount anticipated, by January of 1968 enough sponsorship was obtained to initiate the research program on a reduced scale.

During the time interval between submission of Proposal No. 1194 and the establishment of Group Project 2753, in connection with other studies in our laboratories, it became increasingly obvious to us that the nature and amount of individual components of barks varied with the time of year. Accordingly, it was decided to include in the newly established research project a relatively simple evaluation of the effect of season on the bark extractives of the two southern pines, loblolly and slash. Because our studies on other barks had indicated that the greatest yields of crystalline extractives were obtained from March barks, we chose to employ loblolly and slash pine barks collected in late February or early March for our detailed studies. In addition, samples of unbarked bolts of both slash pine and loblolly pine were obtained from the same locality as the March barks every month for a year. These bolts were barked and submitted to the same extractions employed for the March barks to obtain information relative to possible changes in extractives during the year. The loblolly pine bark was generously supplied by the Wood Procurement Department of U.S. Plywood-Champion Papers Inc., Canton, North Carolina, and the slash pine bark was kindly supplied by the Kraft Division of West Virginia Pulp and Paper Company, North Charleston, South Carolina.

Before the large-scale experiments were performed on March barks, preliminary experiments were performed on barks of four southern pine barks, namely loblolly, slash, longleaf, and shortleaf. These barks were supplied by the Cedar Springs, Georgia mill of Great Northern Paper Company in August of 1967.

The present progress report records our experimental results on the several aspects of this research program to date.

EXPERIMENTAL AND DISCUSSION

PRELIMINARY EXTRACTIONS

Samples of loblolly pine, slash pine, longleaf pine, and shortleaf pine barks obtained in August of 1967 from the general area of Cedar Springs, Georgia had been air-dried and reduced to dust in a Wiley mill. Moisture values for these barks were determined, and the samples were stored in polyethylene bags. All four barks were extracted exhaustively with boiling water, and the extracts obtained were concentrated under reduced pressure for total solids determination. Results obtained are given in Table I.

TABLE I

HOT WATER EXTRACTION OF SOUTHERN PINE BARKS

Pine Bark	Water Extractives, %
Loblolly	15.8
Slash	16.9
Longleaf	11.1
Shortleaf	14.0

Inasmuch as subsequent studies on a larger scale were to be limited to loblolly and slash pine barks, preliminary studies on the fractionation of these water extracts from August pine barks werealso limited to these two species. The preliminary fractionation of the two species bark extractives were performed separately and will be discussed in order. The loblolly extractives were investigated first.

LARGER SCALE EXTRACTION OF LOBLOLLY PINE BARK

A sample of loblolly pine bark dust containing 1,500 grams ovendry solids was divided and placed in two stainless steel pails. Each half was covered with 10 liters of water, stirred while heating to boiling, and filtered through cloth. The residues were squeezed dry and extracted again with boiling water in the same manner. The total extract, amounting to approximately 36 liters, was concentrated in a vacuum circulating evaporator to a volume of approximately 4 liters. This concentrated solution contained considerable solids. Some Celite filter aid was added, and the mixture was filtered through a pad of Celite to yield a clear brown solution. This solution was concentrated further in the vacuum circulating evaporator to 1200 ml. volume, and this concentrated solution contained 79.9 grams of solids, amounting to 5.33% on the ovendry bark solids basis. The concentrated solution was extracted exhaustively with ethyl acetate in a nonagitated liquid-liquid extractor.

The mixture of precipitate and Celite from the above filtration was allowed to air dry. The dried mixture was powdered and extracted exhaustively in a Soxhlet extractor with ethyl acetate. The two ethyl acetate extracts were combined and evaporated to dryness in a vacuum rotary evaporator to yield 34 grams of dark amber sirup.

Polyamide Chromatography

A large polyamide column was prepared by pouring a slurry of polyamide powder [POLYAMIDE WOELM, manufactured by M. Woelm, Eschwege, Germany] in water into a glass column 50 mm. in diameter and 120 cm. in length and allowing the water to drain by gravity. More slurry was added in batches until the packed column reached a height of 80 cm.

The entire ethyl acetate extract was dissolved in warm tetrahydrofuran, and the resulting solution was treated with stirring with dry polyamide powder until a semidry meal was obtained. The solvent was allowed to evaporate, and the dry material was placed on top of the polyamide column in which the water was allowed to drain until its level was even with the top of the polyamide. The dry weight of the ethyl acetate extractives obtained after evaporation of the tetrahydrofuran was 26 grams. The column was filled with water, and elution with water was initiated, collecting 210-ml. samples in the eluate. After collecting 304 fractions of aqueous eluate, the eluting solvent was changed to 10% ethanol, after 332 fractions, it was changed to 20% ethanol, and after 408 fractions, it was changed to 95% ethanol. After 460 fractions were collected, the column was extruded and extracted with hot 95% ethanol to obtain Fraction 461. Each fraction was evaporated to dryness under reduced pressure, weighed, and monitored by thin-layer chromatography on silica gel plates, developed in 4:1 chloroform-methanol, sprayed with 50% sulfuric acid, and heated at 105°C. as described in a previous publication (1). As a result of the thin-layer chromatographic monitoring, the eluate fractions were grouped as shown in Table II. The first five eluate fractions were discarded.

The recovery of material from the polyamide chromatogram of Table II amounted to 91.1% of the actual solids applied to the column.

Because of the preliminary nature of this experiment, a complete fractionation of the individual fractions of Table II was not performed. However, during the processing noted above, several components were noted and identified.

TABLE II

POLYAMIDE CHROMATOGRAPHY OF ETHYL ACETATE-SOLUBLE PORTION OF HOT WATER EXTRACTIVES OF LOBLOLLY PINE BARK[a]

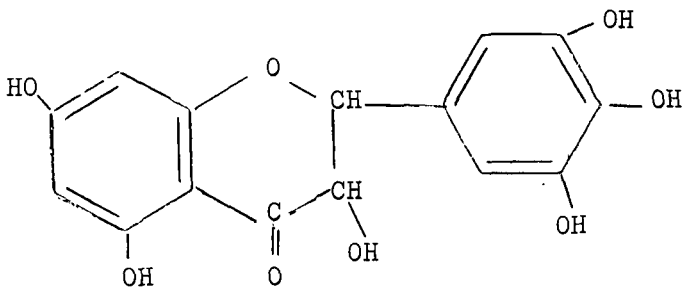
Fraction	Eluate Fractions	Yield, g.
A	6 - 9	3.58
B	10 - 13	1.30
C	14 - 16	0.59
D	17 - 21	0.65
E	22 - 50	2.61
F	51 - 75	0.73
G	76 - 100	0.59
H	101 - 190	1.79
I	191 - 235	1.81
J	236 - 307	1.00
K	308 - 325	0.39
L	326 - 367	0.93
M	368 - 385	0.67
N	386 - 416	3.18
O	417 - 432	0.85
P	433 - 460	0.91
Q	461	2.02
		23.60
	Discarded eluate fractions 1 - 5	0.09
		23.69

[a]Actual thin-layer chromatographic results are available to individual cooperators of Group Project 2753 upon request.

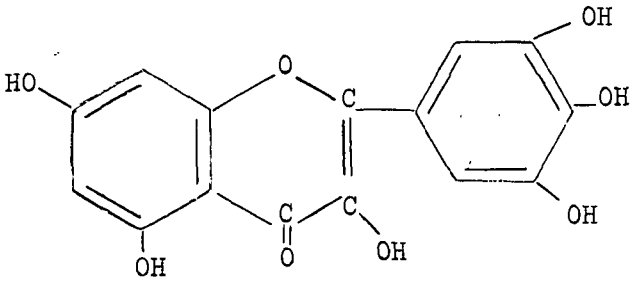
Isolation and Identification of Dihydromyricetin

During the concentration under reduced pressure of eluate Fractions 344 through 392, colorless crystals separated. These eluate fractions comprise all of Fraction M and parts of Fractions L and N of Table II. The crystals were filtered and recrystallized from water in the presence of decolorizing carbon. The resulting crystals melted at 240-260°C. and proved to be a hydrate containing 14.6% water.

Reaction with chlorine water followed by sodium sulfite produced an intense red color suggesting a vicinal trihydroxybenzene structure. In addition, reaction with magnesium and hydrochloric acid gave a strong red-violet coloration characteristic of flavanones, flavonols, and flavanonols. The color reactions suggested dihydromyricetin (3,3',4',5,5',7-hexahydroxyflavanonol) (I) originally isolated from the leaves of Ampelopsis meliaefolia and called ampelopsin by Kotake and Kubota (2). Subsequently, Hergert (3) found this compound in the bark of lodgepole pine (Pinus contorta), but could not isolate the material in its pure form. Instead, he demonstrated its existence by converting it to its dehydrogenated product, myricetin (II).



(I)



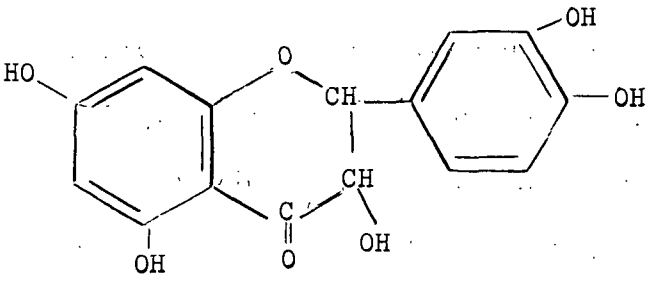
(II)

A sample of authentic dihydromyricetin in its impure form was obtained from Dr. Hergert of the Olympic Research Division of Rayonier Incorporated at Shelton, Washington. This crude product was purified by polyamide chromatography to give a product identical with the compound isolated from loblolly pine bark as determined by infrared spectra, color reactions, and melting point. Finally, our compound was boiled with a solution of 2 grams of sodium bisulfite in 10 ml. of water to convert it to myricetin by the procedure employed by Kurth (4) for converting dihydroquercetin to quercetin. Shortly after boiling commenced, the solution turned yellow, and after 10 minutes, fine yellow needles separated. After 45 minutes of boiling under reflux, the needles were filtered, washed with water, and dried to give yellow needles melting at 350-355°C. with decomposition and not depressing a mixed melting point with authentic myricetin. The infrared spectrum of the yellow needles was identical with that of myricetin.

While exploring the possible structure of the unknown colorless crystals, it was noted that its infrared absorption spectrum was identical with that of an unidentified flavonoid compound isolated by Kinsley and Pearl (5) from the leaves of the quaking aspen (Populus tremuloides).

Isolation and Identification of Dihydroquercetin

Concentration of eluate Fractions 411 through 414 produced colorless crystals. These were filtered and recrystallized from water to give colorless stout needles melting at 240-241°C. and not depressing a mixed melting point with authentic dihydroquercetin. The infrared spectrum was identical with that of authentic dihydroquercetin. Thin-layer chromatography indicated that eluate Fractions 385 through 416 contained dihydroquercetin (III).



(III)

Isolation and Identification of Fatty Acids

Fraction O comprising eluate Fractions 417 through 432, upon evaporation to dryness, left a waxy crystalline residue weighing 0.85 g. and melting at 58-64°C. The crude product appeared to be a mixture of fatty acids. It was recrystallized from methanol to yield 0.21 g. of colorless solid melting at 60-64°C. which was still impure. The crystalline product was labeled Fraction O-1, and the evaporated filtrate from crystallization was labeled Fraction O-2.

Gas chromatography of fatty acids. Both Fractions O-1 and O-2 were subjected to analysis by gas chromatography. The fractions were converted to trimethyl silyl derivatives by reaction with REGISIL (bis-trimethylsilyl trifluoroacetamide obtained from Regis Chemical Company, 1101 North Franklin St., Chicago, Illinois 60610) and chromatographed in an Aerograph Model 202 gas chromatograph with thermal conductivity detector on a 5 ft. by 1/4-inch column of 5% silicone SE-30 on 60-70 mesh ANAKROM ABS (silanized acid-washed and base-washed diatomaceous earth support obtained from Analabs, Inc., 80 Republic Drive, North Haven, Conn. 06473). Quantitation was obtained from integrator readings obtained from known compounds processed under the same conditions.

Weighed samples were dissolved in measured amounts of dry tetrahydrofuran and treated with measured amounts of REGISIL. Reaction is essentially instantaneous. The reaction mixture was chromatographed directly under isothermal conditions at 250°C. with helium carrier gas rate of 75 ml. per minute. The results of these chromatograms are shown in Table III which gives all of the peaks obtained, the yield of each peak component, the total yield in Fraction 0 of each peak component, and the identity of several of the peak components.

Thus, it appears that stearic acid is the chief component of Fraction 0, and other components are fatty acids of known or unknown structure. The two components in amount next to the stearic acid are the last two items of Table III which were not identified in this preliminary experiment. The apparent loss of 83.3% of the components of Fraction 0 is probably due to components which do not form trimethylsilyl derivatives or to trimethylsilyl derivatives which are not volatile under the conditions extant.

Gas Chromatography of All Combined Fractions from the Polyamide Chromatogram

Each of the fractions of Table II was subjected to reaction with the REGISIL reagent and to gas chromatography under temperature-programmed conditions. This was necessary because of the unknown type of components in the several combined fractions. The same instrument and column was employed, but in this experiment the oven temperature at the start was 100°C. and was programmed to 250°C. at the rate of 6° per minute. The preparation of the trimethylsilyl derivatives was the same in all instances, but the solvent employed was different because of solubility problems. Fractions A through C were dissolved in pyridine, Fractions D through G in tetrahydrofuran, and Fractions H through P were dissolved in dimethylformamide. Fraction Q was insoluble and was not chromatographed.

TABLE III

ISOTHERMAL GAS CHROMATOGRAPHY OF FATTY ACID FRACTIONS 0-1 AND 0-2

Peak No.	Retention Time, min.	Yield, mg. Fraction 0-1	Yield, mg. Fraction 0-2	Yield, % of Fraction 0	Component
1	0.7		4.13	1.0	
2	1.9		1.37	0.3	
3	2.1	0.22	17.87	4.4	Palmitic acid
4	2.6	0.22	5.50	1.4	
5	3.1		1.37	0.3	
6	3.6	45.84	166.38	50.8	Stearic acid
7	5.0		1.02	0.2	
8	5.4	0.11		0.1	
9	5.9	1.20	5.15	1.5	Arachidic acid
10	7.1	0.27	1.37	0.4	
11	9.2	0.27		0.1	
12	10.2	0.17		0.1	Behenic acid
13	11.8	0.06		0.1	
14	13.5	8.00	1.02	1.9	n-Tricosanoic acid
15	15.0	4.22		1.0	
16	17.5	0.92		0.2	
17	21.0		14.09	3.3	
18	22.8	4.60		1.1	
19	25.5	3.03	30.91	8.1	
20	29.1		29.22	7.0	
			Total Recovery	83.3	

The results of these individual chromatograms are combined in Tables IV, V, and VI. In these tables, the results are given as retention times and peak heights. Because of the completely unknown nature of the individual components, integrator values could not be standardized for quantitative results. Instead, it should be noted that the product of peak height and retention time gives a relative index of the amount of product associated with the peak for an isothermal chromatogram. In the case of a programmed chromatogram, as given in Tables IV, V, and VI, the relationship is certainly not linear, and the products of these two values are gross approximations.

Fractions J through M were also subjected to isothermal gas chromatography at 250°C. under the conditions noted for Table III. Under these conditions the last five peaks had the following retention times: Peak 13, 16.5 minutes; Peak 14, 17.2 minutes; Peak 15, 23.9 minutes; Peak 16, 24.7 minutes; and Peak 17, 27.3 minutes.

It is apparent from Tables IV, V, and VI that the several fractions of the loblolly pine extractives comprise a great many individual components, and a few of these components are present in substantial quantity. In this preliminary experiment, no attempt was made to determine the structure of most of these components obtained by gas chromatography of the trimethylsilylated fractions. Gas chromatography determines the components of a mixture which are volatile under the conditions of the chromatogram, irrespective of their structure. Thus, under the conditions employed in this preliminary experiment, peaks were obtained for trimethylsilyl derivatives of hydroxy- and carboxy-compounds as well as from original components of the fractions which were volatile without derivatization. Thus, some fractions were submitted to programmed gas chromatography under the same conditions without previous treatment with REGISIL.

TABLE IV

PROGRAMMED GAS CHROMATOGRAPHY OF FRACTIONS A THROUGH I

Peak No.	Retention Time, min.	Peak Height for Fraction, units								
		A	B	C	D	E	F	G	H	I
1	2.2					2		2	3	
2[a]	3.3	8			1	2	2	2	3	
3	3.6	4	15	10	5	6	5	5	4	
4	4.2	3	6	4						
5	4.7					1	2			
6	5.2								3	2
7	6.1		1	1						
8	7.2	1			1	1	3			
9	8.0	1	2	1	2					
10	8.7	72	7	7	2	1				
11	9.0	4								
12	9.7	1	1	1		1				
13	10.1	1								
14	10.7	6	4	3						
15	11.3	1	4	1	1					
16	11.7	12	2	2						
17	12.3	3	3	2	4	9	6	10		1
18	12.6	2			1	4				
19	13.3	14	1		2	4	7	31	5	
20	13.5						6			
21	13.8	1	1							
22	14.3	2	1	2						
23	14.5					7		16	1	
24	14.8	11	1							
25	14.9								5	1

See end of table for footnote.

TABLE IV (Continued)

PROGRAMMED GAS CHROMATOGRAPHY OF FRACTIONS A THROUGH I

Peak No.	Retention Time, min.	Peak Height for Fraction, units								
		A	B	C	D	E	F	G	H	I
26	15.2	6	1							
27	15.6		1	9	3					
28	15.8								6	
29	15.9	5	1	8	9					
30	16.9	8	2	13	6	1	24	5		
31	17.5	13	3	1	3	2	2	46	22	2
32	18.3	8								
33	18.7	28	28	8	1	2				
34	19.2	23	5	2						
35	20.0		15	12	1	5				
36	20.5	30	5	1	1	2		2	3	
37	21.3	19	15	5	1			1	1	
38	21.8	11			1					
39	22.3		1	5					2	2
40	23.0	5	4	3						
41	23.5		3	2	2			1		
42	23.9			1	1	1				
43	25.3		3							
44	25.8	1	4							
45	26.8		1	1	1	3			2	17
46	28.0			1	1		6			
47	28.7						1			
48	29.5	2		1						
49	30.4	2								
50	31.3	1	1	5	5	2	5		5	4
51	32.5						1			
52	33.6		1				2			
53	37.1			3						
54	38.0		2							
55	42.5		2	4	2					

[a] When there is a break in consecutive fractions for a particular peak or when the peak height goes through a minimum, the components responsible for the peak in different fractions are probably different.

TABLE V

PROGRAMMED GAS CHROMATOGRAPHY OF FRACTIONS J THROUGH M

Peak No.	Retention Time, min.	Peak Height for Fraction, units J	K	L	M
1	3.0	3	2	2	1
2	3.4	2	2		
3	4.2	2	1	1	1
4[a]	5.7	9	6	17	1
5	6.2		28		
6	8.6		2		
7	11.9			1	
8	12.2				1
9	12.8		1		
10	13.4		1		
11	15.8		1	1	
12	20.1			2	
13	39.8			3	5
14	43.1		77	26	5
15[bc]	48.9		2	5	9
16[c]	50.3			10	
17[c]					21

[a] This peak corresponds with Peak No. 6 of Table IV.

[b] This peak is the trimethylsilyl derivative of dihydromyricetin. Most of the dihydromyricetin had been removed as crystals from these fractions.

[c] This is a very broad peak. Therefore, the peak heights recorded indicate major components.

TABLE VI

PROGRAMMED GAS CHROMATOGRAPHY OF FRACTIONS N., O,. AND P

Peak No.	Retention Time, min.	Peak Height, units N	O	P	Matching Peak in Table IV
1	3.2		2		
2	3.8	1	1	1	3
3	5.6	2		2	4
4	12.3		2	9	
5	14.8			1	
6	15.6	1	3	1	
7	18.0			1	
8	22.1	3	1	1	
9[a]	23.0	6	6	2	
10	24.2	2	3	1	
11	25.2	2	2	2	
12[b]	26.2	10	55	5	
13[c]	28.2	1	4	2	
14[d]	30.3	2	1		
15	32.0		3		
16	36.5	2	1	1	
17	37.8		6	3	
18	39.5			1	
19	41.5	7			14
20	43.6			1	
21	45.8	15	1		15
22	49.5	8			17

[a] This is the trimethylsilyl derivative of palmitic acid.

[b] This is the trimethylsilyl derivative of stearic acid.

[c] This is the trimethylsilyl derivative of arachidic acid.

[d] Peaks 14 through 22 are broad peaks.

When Fraction A was chromatographed directly, a total of 24 peaks was obtained, but none of these matched peaks noted in Table IV. However, when Fraction F was chromatographed directly, a total of 13 peaks was obtained and 7 of these peaks were identical with those of Table IV. These were Peaks 5, 8, 17, 19, 20, 46, and 47. Although some of these peaks were

present in the trimethylsilylated chromatogram of Fraction A, the component responsible for the peaks must have been different.

IMPORTANT NOTE: The results reported in such tables as III-VI and all succeeding gas chromatographic tables are condensed from large chart papers. These original gas chromatograms are on file and are available to any of the cooperators of Group Project 2753 for inspection upon request.

LARGER-SCALE EXTRACTION OF SLASH PINE BARK

A 1500-g. (ovendry basis) sample of slash pine bark dust was processed in the manner described in detail for the loblolly pine bark dust. The yield of water extractives in this instance amounted to 5.4% of the original bark, and the ethyl acetate-soluble portion in its airdry condition amounted to 33% of the water extractives.

Polyamide Chromatography of Slash Pine Extractives

The ethyl acetate extractives were dissolved in tetrahydrofuran and absorbed on polyamide as described previously to give a dry weight of ethyl acetate extractives of 24 grams. The absorbed extractives were placed on top of a polyamide column as before and eluted with water and ethanol dilutions as follows: Fractions 1 through 75, water; Fractions 76 through 154, 20% ethanol; Fractions 155 through 204, 50% ethanol; Fractions 205 through 238, 95% ethanol; and Fraction 239, hot 95% ethanol extract of extruded column. The individual eluate fractions were concentrated and monitored by thin-layer chromatography as detailed for the loblolly bark experiment. On the basis of the monitoring chromatograms, the eluate fractions were grouped and combined in accordance with the data of Table VII. The first 800 ml. of eluate was discarded before the first eluate fraction was collected.

TABLE VII

POLYAMIDE CHROMATOGRAPHY OF ETHYL ACETATE-SOLUBLE PORTION OF HOT WATER EXTRACTIVES OF SLASH PINE BARK

Fraction	Eluate Fractions	Yield, g.
A	1 - 7	4.74
B	8 - 12	1.11
C	13 - 17	0.78
D	18 - 25	0.75
E	26 - 49	1.53
F	50 - 62	0.50
G	63 - 84	0.57
H	85 - 102	1.15
I	103 - 134	0.91
J	135 - 162	1.43
K	163 - 166	1.52
L	167 - 188	1.65
M	189 - 238	2.24
N	239	4.12
		23.00

The recovery of material from the polyamide chromatogram of Table VII amounted to 96%. Unlike the eluate fractions from the loblolly bark extractives chromatogram, none of the fractions from the slash pine bark extractives chromatogram deposited crystals upon concentration and, therefore, no components were isolated and identified from these eluate fractions.

Gas Chromatography of Combined Fractions from the Polyamide Chromatogram

All combined fractions from the polyamide chromatogram of the slash pine bark extractives were reacted with REGISIL and subjected to temperature-programmed gas chromatography exactly as detailed for the loblolly pine bark experiment. In this slash pine study, all fractions except M were dissolved in dimethylformamide for trimethylsilylation with REGISIL. Fraction M was dissolved in tetrahydrofuran. Fraction N was insoluble in all solvents, so it could not be gas chromatographed.

The results of the individual chromatograms are combined in Tables VIII, IX, and X.

The data of Tables VIII, IX, and X indicated that several of the fractions contained certain compounds in relatively high concentration. These were Fractions F, G, H, I, and J which were chromatographed under isothermal conditions at 250°C. as noted previously for loblolly pine bark fractions. Under these isothermal conditions, important peaks in Tables VIII and IX had retention times as noted in Table XI.

Our experience demonstrates that these 11 major components can be separated and isolated from their REGISIL reaction products relatively easily by preparative chromatographic procedures. However, because of the preliminary nature of this experiment, fractions were stored for future comparison studies.

TABLE VIII

PROGRAMMED GAS CHROMATOGRAPHY OF SLASH PINE BARK FRACTIONS A THROUGH G

Peak No.	Retention Time, min.	Peak Height for Fraction, units						
		A	B	C	D	E	F	G
1	2.3							2
2	2.7				3	3	1	
3	3.0	2						
4	3.2	2						
5	4.0	8	2	2	2	2	2	2
6	5.4	4						
7	5.6				1	2		
8	5.7	2						
9	6.1							1
10	6.9				2			
11	7.5					6		
12	8.3	17	1	1				
13	8.4							1
14	8.8		1					
15	9.1	1	2	1	1			
16	9.3						2	
17	10.5	2	1	3	4	2		
18	11.0				1			2
19	11.1	1	1		1			
20	11.6	5						
21	12.0	2	1	1	1	1		
22	12.5	2	8	28	10	9	12	
23	12.8				12		1	21
24	13.3	5	1	2	21	2		
25	13.7				4	7	91	1
26	14.2	3	1					
27	14.6		2	2	1			
28	14.9		2	2	7	9		
29	15.1						3	48
30	15.3	4	5					

TABLE VIII (Continued)

PROGRAMMED GAS CHROMATOGRAPHY OF SLASH PINE BARK FRACTIONS A THROUGH G

Peak No.	Retention Time, min.	Peak Height for Fraction, units						
		A	B	C	D	E	F	G
31	15.6					9	1	
32	15.9	2	1	14	4	2	1	1
33	16.4	1	6	2	3	2		
34	17.3		1	3	1	1	38	1
35	17.5	5	1	2	16	3		
36	18.3			5				
37	18.7	3	15	2	2	3	1	
38	19.1	6	1	70	11			
39	19.2							100+
40	19.8	5	2	2	5	16	1	1
41	20.6		3	2	8	1	2	
42	20.8				3	1		1
43	21.1	50						
44	21.5	3	17	15				10
45	22.1					1	11	4
46	22.3	7	13	1				
47	23.0		5					
48	23.3	1	4					
49	23.6				3	1		
50	24.1		1	1				
51	25.1		2	1	1	2		
52	25.7	1	3	4		2		
53	26.6	7	1		1	1	1	
54	27.2		2					
55	27.6		6					
56	28.2						3	
57[a]	28.7		1					
58	29.3	1	1	1		1		
59	30.8					1	1	
60	31.6		2	1				

See end of table for footnote.

TABLE VIII (Continued)

PROGRAMMED GAS CHROMATOGRAPHY OF SLASH PINE BARK FRACTIONS A THROUGH G

Peak No.	Retention Time, min.	Peak Height for Fraction, units						
		A	B	C	D	E	F	G
61	33.8			1	1	1	1	
62	34.8		6					
63	37.6		8					
64	43.3					-	1	5
65	44.6			3	1			
66	48.7					4	2	
67	51.5					2		
68	57.4			2				

[a]Peaks 57 through 68 are broad peaks.

TABLE IX

PROGRAMMED GAS CHROMATOGRAPHY OF SLASH PINE BARK FRACTIONS H THROUGH K

Peak No.	Retention Time, min.	Peak Height for Fractions, units H	I	J	K	Matching Peak in Table VIII
1	3.0		3	2	1	
2	4.2	2	4	6		5
3	6.3		8			
4	8.5		2	2		
5	12.5	6	7	2	1	23
6	13.8			1		
7	14.9	5	1			29
8	15.8		1	2		
9	17.4	1	1	1		34
10	18.7	28	2	2		39
11	19.7	1				40
12	20.1	1	2			41
13	20.7		2	8		
14	21.2	3				42
15	22.0	2	2	1		45
16	22.9	18	100+	1		
17	24.0	1	1	18		
18	26.4		1	4		
19[a]	27.5	1	1			
20	29.2		1	2	3	
21	30.3		1			
22	31.5		1			
23	33.5	2	1			
24	36.4	1				
25	41.6			26	28	
26	45.2	5		2	13	
27	49.2	12		3		
28	55.0		4			

[a]Peaks 19 through 28 are broad peaks.

TABLE X

PROGRAMMED GAS CHROMATOGRAPHY OF SLASH PINE BARK FRACTIONS L AND M

Peak No.	Retention Time, min.	Peak Height, units L	Peak Height, units M	Matching Peak in Table IX
1	4.6	1		
2	7.4		1	
3	9.2		2	
4	12.7	3		5
5	15.0		3	
6	24.0	2	1	
7	25.0	6		
8	26.5	4		
9	27.2	8		
10	27.7	2	1	
11[a]	28.7	2	2	
12	30.1	1		20
13	31.1		1	
14	34.0		2	
15	37.0		1	
16	42.5	4	1	
17	45.9		1	
18	47.0	5		26
19	48.8		1	
20	71.8		2	

[a]Peaks 11 through 20 are broad peaks.

TABLE XI

ISOTHERMAL GAS CHROMATOGRAPHY OF SELECTED SLASH PINE BARK FRACTIONS

Peak from Table VIII	Retention Time, min.	Peak from Table IX	Retention Time, min.
25	0.7	16	2.1
34	1.0	17	2.5
39	1.2	25	17.8
44	1.5	26	20.8
45	1.7	27	24.5
64	18.0		

PRELIMINARY SOLVENT EXTRACTIONS

Having found fatty acids as such a major component of the bark extractives isolated after polyamide chromatography of the ethyl acetate-soluble portion of the hot water extractives, an attempt was made to extract fatty acids and similar materials in a mineral solvent prior to hot water extraction. In order to determine which solvent to employ for the preliminary solvent extraction, three identical samples of the same loblolly bark used in this study were extracted in individual Soxhlet extractors with petroleum ether (b.r. 30-50°C.), hexane, and heptane. After exhaustive extraction, the bark dust residues were extracted with water in the same Soxhlet apparatus. Yield data for these extractions are given in Table XII.

TABLE XII

SOLVENT EXTRACTION OF LOBLOLLY PINE BARK DUST

Solvent	Extract Yield, %	Subsequent Water Extract, %	Total Yield, %
Petroleum ether (30-60°C.)	3.7	4.7	8.4
Hexane	2.5	5.8	8.3
Heptane	2.9	7.0	9.9

Gas Chromatography of Solvent Fractions

The individual fractions were submitted to temperature-programmed gas chromatography under conditions identical with those employed for the earlier fractions from the polyamide chromatogram. Results of the gas chromatograms are given in Table XIII.

TABLE XIII

PROGRAMMED GAS CHROMATOGRAPHY OF PETROLEUM ETHER EXTRACTIVES OF LOBLOLLY PINE BARK

Peak No.	Retention Time, min.	Peak Height, units		
		Petr. Ether	Hexane	Heptane
1	2.3	10	3	2
2	2.9	12	1	1
3	3.4	5	3	4
4	3.9	1		
5	4.2	2		
6	4.8	2		
7	5.3	1	1	1
8	5.7	1		
9	6.1	1		
10	6.3		1	1
11	6.6	2	1	1
12	8.5		2	2
13	9.7	3	4	6
14	10.7	2	1	1
15[a]	16.0	3	3	5
16	16.7	1	1	1
17[b]	17.7	7	10	12
18	18.0	2	2	2
19	18.7	6	7	9
20	19.3	27	27	33
21[c]	19.7	4	6	6
22	20.5	1	1	1
23[d]	21.2	2	2	2
24	21.7	2	2	3
25	22.7	2	2	3
26	24.9	2	3	3
27	25.7	1	1	1
28	26.9	2	3	3
29	28.2		2	

[a] Corresponds with the peak for trimethylsilylated palmitic acid.

[b] Corresponds with the peak for trimethylsilylated stearic acid.

[c] Corresponds with the peak for trimethylsilylated arachidic acid.

[d] Peaks 23 through 29 are broad peaks.

The individual fractions were then gas chromatographed under isothermal conditions at 250°C. exactly as described previously. Under these conditions, the first 11 peaks came off with the solvent and could not be determined. The remaining peaks were correlated with those of Table XIII. As in the case of the temperature-programmed runs of Table XIII, the isothermal chromatograms of the three solvent fractions were essentially the same. Accordingly, only that of the petroleum ether extractives have been reported in Table XIV.

TABLE XIV

ISOTHERMAL GAS CHROMATOGRAPHY OF PETROLEUM ETHER EXTRACTIVES OF LOBLOLLY PINE BARK

Peak No.	Retention Time, min.	Peak Height, units	Matching Peak in Table XIII
1	0.7	10	12
2	0.9	6	13
3	1.6	1	14
4[a]	2.0	7	15
5	2.5	1	16
6[b]	3.1	12	17
7	3.7	1	18
8	4.4	9	19
9	4.9	28	20
10[c]	5.3	6	21
11	6.3	1	22
12	7.2	3	23
13	7.7	2	24
14	9.0	2	25
15	12.0	3	26
16	13.7	1	27
17	15.0	2	28

[a] Corresponds with the peak for trimethylsilylated palmitic acid.
[b] Corresponds with the peak for trimethylsilylated stearic acid.
[c] Corresponds with the peak for trimethylsilylated arachidic acid.

The present experiment demonstrated that the fatty materials could be isolated simply by preliminary extraction with a hydrocarbon solvent. Of the three solvents tried, the petroleum ether (b.r. 30-60°C.) appeared to remove the largest amount of extractives. The poor yield of water-soluble material in the three individual extractions of this experiment was attributed to the fact that water extraction was performed in a Soxhlet apparatus in which the bark dust is actually extracted with warm water from the condensation of boiling water in the condenser. In the previous water extractions, the bark dust was extracted with boiling water which apparently extracted considerably more material.

Accordingly, it was decided for subsequent experiments to extract the bark dust with petroleum ether, allow to dry, and then extract with hot water in the stainless steel buckets as noted in the first large-scale extraction.

While these last experiments were in progress, new barks were obtained in quantity. Further work on the preliminary fractionation of the older barks was discontinued so that the new barks could be investigated.

LARGE-SCALE INVESTIGATION OF MARCH LOBLOLLY PINE BARK

On March 7, 1968 we received a large sample of fresh loblolly pine bark from U.S. Plywood-Champion Papers Inc. Carolina Division mill in Canton, North Carolina. The fresh bark had been shipped from Canton on February 27, 1968. The bark was allowed to airdry and was then reduced to dust in a Wiley mill. The airdry bark containing 10.0% moisture was stored in the cold room in polyethylene bags.

Extractions of Bark Dust

In order to obtain a preliminary fractionation of the extractives of loblolly pine bark, the airdry bark dust was extracted successively with petroleum ether, water, ether, and 95% ethanol. The total bark processed amounted to 7,960 grams ovendry. This was processed in four identical batches. Each batch was extracted exhaustively with petroleum ether in a large Soxhlet apparatus. The residual bark was allowed to airdry, and after all petroleum ether had evaporated, the bark was processed with boiling water exactly as detailed for the larger-scale hot water extraction of the August loblolly pine bark dust. After the water extraction, the bark was airdried and then extracted successively in the large Soxhlet apparatus with ether and with 95% ethanol. All extracts were concentrated under reduced pressure to relatively small volumes for solids determination. Yields of solids on the basis of the original ovendry solids of the starting bark dust are reported in Table XV.

TABLE XV

EXTRACTIONS OF MARCH LOBLOLLY PINE BARK DUST

Solvent	Yield Extractives, % original bark
Petroleum ether (b.r. 30-60°C.)	2.4
Water	5.1
Ether	1.3
95% Ethanol	0.7
Total	9.5

The hot water extract was then extracted with ethyl acetate as described earlier to obtain 33.3% ethyl acetate extractives corresponding with 1.7% of the original bark solids.

Polyamide Chromatography of Ethyl Acetate Extractives

The ethyl acetate extractives were dissolved in tetrahydrofuran, absorbed on polyamide, and allowed to airdry. The weight of ethyl acetate extractives absorbed on the polyamide was 32.0 grams. This extractives-polyamide mixture was placed on top of a column of polyamide 50 mm. in diameter and 80 cm. in length and developed with water and dilutions of ethanol in the manner detailed for the earlier polyamide chromatograms as follows: Fractions 1 through 80, water; Fractions 81 through 110, 10% ethanol; Fractions 111 through 200, 20% ethanol; Fractions 201 through 305, 50% ethanol; Fractions 306 through 350, 95% ethanol; and Fraction 351, hot 95% ethanol extract of the extruded column.

As in the past, each fraction was evaporated to dryness on the vacuum rotating evaporator, weighed, and monitored by thin-layer chromatography. On the basis of the monitoring thin-layer chromatograms, the eluate fractions were grouped and combined in accordance with the data of Table XVI.

The total recovery of material from the column amounted to 96% of the solids applied to this polyamide chromatographic column. As in the case of the August slash pine bark fractions, none of the fractions of this chromatogram of March loblolly pine bark extractives deposited crystals upon concentration to a small volume.

Gas Chromatography of Combined Fractions

All combined fractions noted in Table XVI were dissolved in a solvent, reacted with REGISIL, and subjected to temperature-programmed gas chromatography exactly as described for the August loblolly pine bark experiment. Fractions L-A through L-N were dissolved in dimethylformamide, Fraction L-O was dissolved

TABLE XVI

POLYAMIDE CHROMATOGRAPHY OF ETHYL ACETATE-SOLUBLE PORTION OF HOT WATER EXTRACTIVES OF MARCH LOBLOLLY PINE BARK

Fraction	Eluate Fractions	Yield, g.
L-A	1 - 2	0.45
L-B	3 - 17	5.97
L-C	18 - 21	0.52
L-D	22 - 28	0.75
L-E	29 - 40	1.38
L-F	41 - 62	1.45
L-G	63 - 108	1.51
L-H	109 - 126	0.86
L-I	127 - 154	1.03
L-J	155 - 203	1.57
L-K	204 - 219	1.05
L-L	220 - 226	3.32
L-M	227 - 229	0.39
L-N	230 - 266	1.94
L-O	267 - 350	5.93
L-P	351	2.51
		30.63

in tetrahydrofuran, and Fraction L-P could not be dissolved in any solvent. The results of the temperature-programmed gas chromatography of these fractions are given in Tables XVII, XVIII, and XIX. Fraction L-A gave no component peaks, so was not included in the tables.

Investigation of the Petroleum Ether Extractives (Fraction L-PE)

This fraction, representing 2.4% of the original bark solids, was dissolved in dimethylformamide, treated with REGISIL, and gas chromatographed under temperature-programmed conditions reported for previous fractions. Although 22 individual peaks were obtained, the chromatogram was not satisfactory, and certainly not worthy of further study.

The crude fraction was then submitted to direct gas chromatography on an FFAP column under conditions used previously for the chromatography of fatty acid mixtures. These conditions comprised the use of a 5 foot by 1/4 inch column of 20% FFAP on 70-80 mesh Chromosorb W, DMCS (obtained from the Varian-Aerograph Company of Walnut Creek, California) in the Aerograph Model 202 chromatograph with thermal conductivity detectors and with a helium carrier gas flow rate of 75 ml. per minute. The sample was introduced at 110°C., the oven is maintained at 110°C. for 5 minutes, and then programmed to 250°C. at the rate of 10° per minute. Again a number of peaks were obtained, but a majority of the material did not chromatograph.

Hydrolysis of Petroleum Ether Extractives

Accordingly, a sample of these extractives was saponified. An aliquot of Fraction L-PE containing 5.0 g. of solids was evaporated to dryness, covered with 100 ml. of N sodium hydroxide solution, and boiled under reflux for 4 hours.

TABLE XVII

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH LOBLOLLY PINE BARK EXTRACTIVES FRACTIONS L-B THROUGH L-G

Peak No.	Retention Time, min.	Peak Height for Fraction, units					
		L-B	L-C	L-D	L-E	L-F	L-G
1	3.2	1					
2	4.4	6	1	1	1	1	
3	4.6	4				1	1
4	5.3			4			
5	7.1		1		1		
6	8.7		2	1	1	1	1
7	9.7	4					
8	10.2			6	2		
9	11.1		13				
10	11.8		39	3	1		
11	12.8				3	3	2
12	13.0		2	4			
13	13.4		1	18	2		
14	14.3	2	1			1	1
15	14.7		1				
16	15.3	5	8	2	17	20	11
17	15.6			2	2	2	
18	16.5	6	13	1	2	1	
19	17.1		2	2			
20	17.8	2	1	1	2	35	1
21	18.5	1	2	10	1		
22	18.9				1	100+	56
23	19.2	5	32				
24	19.6	3	29	23			
25	20.0	3	3	15			
26	20.2			3	16	2	1
27	20.7			2	3		
28	21.0			6	1	2	1
29	21.3	4		3			
30	21.6					23	9

TABLE XVII (Continued)

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH LOBLOLLY PINE BARK EXTRACTIVES FRACTIONS L-B THROUGH L-G

Peak No.	Retention Time, min.	Peak Height for Fraction, units					
		L-B	L-C	L-D	L-E	L-F	L-G
31	21.9	3	1		1		
32	22.4					11	5
33	23.0	1					
34	23.4				1	1	8
35	23.9	8					
36	24.2	6	1				
37	24.5				1	2	
38	24.8		1	1	1		1
39	25.6	3	1		1		
40	26.0				2	3	
41	26.3	2					
42	26.8	12	2	1			
43[a]	27.0				1	2	1
44	27.5	4					
45	28.0			1	1		
46	28.6	5					
47	28.7					1	1
48	29.2					1	
49	29.6						
50	30.8	1					
51	31.1				2	1	
52	32.5			1	2		
53	33.0	2					
54	34.2	4	1	1			
55	36.5			1			
56	45.9		3	2			
57	46.5						1
58	49.2				2		1
59	52.0				2		

[a] Peaks 43 through 59 are broad peaks.

TABLE XVIII

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH LOBLOLLY PINE BARK EXTRACTIVES FRACTIONS L-H THROUGH L-K

Peak No.	Retention Time, min.	Peak Height for Fractions, units L-H	L-I	L-J	L-K	Matching Peak in Table XVII
1	3.0	3	72	36	3	
2	4.1	2	1	3	1	3
3	8.4	I	1	2	1	6
4	12.5	1	3	1	1	11
5	17.5	1				
6	18.1			1		
7	18.8	1	5	1		22
8	19.3				1	
9	20.2	16	2			26
10	20.8		20	26	1	
11	21.6		1	2		
12	22.6	4		1		32
13	23.2	100+	24	2	2	34
14	24.2	1	45	35	1	
15	24.7	2				38
16	25.5			1		
17[a]	30.6		1			
18	31.8		2	1	2	
19	34.0		2	1	1	
20	35.8			1		
21	36.6	4	1			
22	38.8				2	
23	40.5		1	2	1	
24	43.9		1	46		
25	44.8	5		2	4	
26	49.0	17	1			58
27[b]	49.3				20	
28	52.5		3			
29	54.4	1	7			

[a] Peaks 17 through 29 are broad peaks.

[b] This component was identified as dihydromyricetin by identity of thin-layer and isothermal gas chromatograms with authentic material.

The mixture was cooled and extracted with ether. The ether was evaporated to yield a neutral fraction, L-PE-N, containing 1.20 g. of solids. The aqueous raffinate was acidified carefully with dilute sulfuric acid and extracted again with ether. This ether extract was evaporated to yield the acidic fraction, L-PE-A, containing 2.54 g. of solids. Finally, the acid aqueous raffinate was neutralized with barium carbonate, filtered to remove barium sulfate, and concentrated to give the water-soluble acid fraction, L-PE-W.

TABLE XIX

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH LOBLOLLY PINE BARK EXTRACTIVES FRACTIONS L-L THROUGH L-O

Peak No.	Retention Time, min.	Peak Height for Fractions, units				Matching Peak in Table XVIII
		L-L	L-M	L-N	L-O	
1	4.0	1	1	1	1	2
2	8.3			2		
3	12.8	3	20	8	4	4
4	15.3			1	1	
5	22.4			13		
6	23.2				2	
7	23.8		2	3	1	
8	24.3				1	
9	26.0	1	2	5	6	
10	27.5	2	2			
11	27.8				1	
12	29.0	2	9	1		
13	29.9	2	3			
14[a]	30.9	1	19	22	1	
15	31.7	1	2			18
16	36.5			1	1	
17[b]	41.7	3	7	3		
18	47.1	30	7	1		27
19	49.3	2				

[a] Peaks 14 through 19 are broad peaks.

[b] This component was identified as dihydroquercetin by identity of thin-layer and isothermal gas chromatograms with authentic material.

Gas chromatography of Fraction L-PE-N. This neutral fraction was trimethylsilylated in the usual manner with REGISIL and gas chromatographed under the same temperature programmed conditions described earlier. The chromatographic results are given in Table XX which also includes a column for the product of peak height and retention time, a value noted earlier to give a relative index of the amount of product associated with the peak.

TABLE XX

PROGRAMMED GAS CHROMATOGRAPHY OF NEUTRAL FRACTION L-PE-N

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time
1	2.6	5	13
2	3.1	3	9
3	4.0	5	20
4	6.4	5	32
5	7.4	2	15
6	11.4	48	548
7	12.0	11	132
8	12.8	1	13
9	13.2	3	40
10	14.2	4	57
11	16.6	5	83
12	23.1	23	532
13	24.8	2	50
14	26.0	3	78
15	26.9	2	54
16	27.5	5	137
17	28.1	2	56
18	29.8	2	60
19	31.4	12	376
20	37.7	66	2,490
21	40.4	1	40
22	45.8	18	825

The trimethysilylated fraction was also chromatographed under isothermal conditions at 250°C. as described previously. These results are given in Table XXI. Table XXI gives the isothermal retention times for the peaks noted in Table XX. These retention times were compared with the isothermal retention times noted for authentic fatty alcohols and acids, and where they correspond the indication is made in Table XXI.

TABLE XXI

ISOTHERMAL GAS CHROMATOGRAPHY OF NEUTRAL FRACTION L-PE-N

Peak of Table XX	Isothermal Retention Time, min.	Corresponding Authentic Component
9	0.7	
10	0.9	
11	1.0	
12	2.3	octadecanol
13	3.1	
14	3.6	oleic and/or linoleic acid
15	4.1	
16	4.3	eicosanol
17	4.8	arachidic acid
18	6.3	
19	7.2	
20	12.6	tetracosanol
21	15.3	
22	20.2	hexacosanol

Thus, the combined data of Tables XX and XXI indicate that the chief component of the neutral fraction obtained upon saponification of the petroleum

ether extractives was tetracosanol, and the important minor products were related long-chain aliphatic alcohols. It is interesting to note that the retention time for the trimethylsilyl derivative of hexadecanol (palmityl alcohol) under the isothermal conditions employed in this experiment is 1.6 minutes. The chromatogram of Fraction L-PE-N was blank at that retention time, and, therefore, contained no hexadecanol whatsoever. However, an important component of the fraction gave a peak at 11.4 minutes under the temperature-programmed conditions, but came over with the solvent under the high temperature isothermal conditions. The nature of this component was not investigated at this stage of the investigation of the petroleum ether extractives. [This component was investigated in connection with a continuing study of the effect of time of year on loblolly bark extractives components and will be reported later.]

To prove the composition of the component giving the exceptionally large Peak Number 20 of Tables XX and XXI, the effluent of Peak Number 20 was collected from several isothermal chromatograms of Fraction L-PE-N. The effluent was dissolved in ether, and the ether solution was shaken with a little water to decompose the trimethylsilyl derivative. The ether layer was removed and evaporated to yield a white waxy solid. An infrared absorption spectrum of this solid indicated conclusively that it was tetracosanol.

Gas chromatography of Fraction L-PE-A. This ether-soluble acidic fraction was trimethylsilylated with REGISIL and submitted to both temperature-programmed and isothermal gas chromatography under the same conditions employed for the neutral Fraction L-PE-N. Data for these chromatograms are given in Tables XXII and XXIII.

TABLE XXII

PROGRAMMED GAS CHROMATOGRAPHY OF ACIDIC FRACTION L-PE-A

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time
1	2.4	2	5
2	2.8	2	6
3	3.6	5	18
4	7.9	1	8
5	12.4	4	50
6	19.3	1	19
7	20.2	3	61
8	22.3	3	67
9	22.7	20	455
10	23.4	51	1,190
11	23.9	2	48
12	25.4-25.7	100+	2,560+
13	26.9	16	431
14	27.3	6	164
15	28.0	60	1,680
16	28.4	15	426
17	28.9	5	145
18	30.3	8	242
19	31.1	8	249
20	31.8	2	64
21	33.0	13	429
22	36.1	2	72
23	39.5	20	740
24	49.6	4	199

The combined results of Tables XXII and XXIII demonstrate that the major component of the ether-soluble acid is oleic and/or linoleic acid along with substantial amounts of some other long-chain fatty acids and a component corresponding with Peak No. 10. It should be noted that stearic acid was not found in this chromatogram.

The finding of free stearic acid as a major component of the extractives of the loblolly pine bark used in the preliminary study of this report (obtained in August, 1967 from Cedar Springs, Georgia) and essentially no stearic acid in the saponified petroleum ether extractives of this March loblolly pine bark led

TABLE XXIII

ISOTHERMAL GAS CHROMATOGRAPHY OF ACIDIC FRACTION L-PE-A

Peak of Table XXII	Isothermal Retention Time, min.	Corresponding Authentic Component
3	0.7	
4	0.8	
5	1.1	
6	1.3	
7	1.5	
8	1.8	
9	2.0	Palmitic acid
10	2.2	
11	2.6	
12	3.1	Oleic and/or linoleic acid
13	4.2	
14	4.5	
15	4.8	Arachidic acid
16	5.3	
17	6.0	
18	7.0	
19	8.7	
20	9.7	
21	11.7	
22	12.6	
23	14.8	Tetracosanoic acid
24	24.5	Hexacosanoic acid

us to a reinvestigation of the nature of the components of the peak in the neighborhood of 26 minutes retention time under the programmed gas chromatography conditions employed in all studies reported in this report.

The fractions in question were rechromatographed along with authentic free fatty acids without REGISIL treatment directly on the FFAP column described previously under the investigation of the petroleum ether extractives (Fraction L-PE) under isothermal conditions at 265°C.

The results for Fraction 0 of the early August loblolly pine bark are given in Table XXIV. [Compared with Table III.] It is apparent that the isothermal chromatogram of Table XXIV will give peaks for more free fatty acids than does that for the trimethylsilylated acid under lower temperature conditions (Table III).

TABLE XXIV

ISOTHERMAL GAS CHROMATOGRAPHY OF FREE ACID FRACTION 0

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
1	1.0	10	10	Heptanoic acid
2	1.8	1	2	Decanoic acid
3	2.6	3	8	
4	2.8	2	6	Dodecanoic acid
5	3.5	1	3	Tridecanoic acid
6	4.4	1	4	Myristic acid
7	4.7	1	5	
8	7.0	2	14	Palmitic acid
9	10.9	15	163	Stearic acid
10	13.2	1	13	Linoleic acid

The results of Fraction L-PE-A of the March loblolly pine bark are given in Table XXV.

TABLE XXV

ISOTHERMAL GAS CHROMATOGRAPHY OF ACIDIC FRACTION L-PE-A AS FREE ACIDS

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
1	1.2	7	8	Octanoic acid
2	2.2	45	99	Undecanoic acid
3	3.1	29	90	
4	3.5	17	60	Tridecanoic acid
5	4.4	3	13	Myristic acid
6	5.5	1	5	Pentadecanoic acid
7	7.0	12	100	Palmitic acid
8	10.3	2	21	
9	10.9	1	11	Stearic acid
10	11.8	15	177	Oleic acid
11	13.4	16	204	Linoleic acid

The isothermal chromatograms of the free fatty acids in Tables XXIV and XXV prove that stearic was in fact the chief component of Fraction 0 from the polyamide chromatography of the Cedar Springs, Georgia sample of August loblolly pine bark extractives without saponification and that the chief components of Fraction L-PE-A comprising the acidic fraction of the saponified petroleum ether extractives of the Canton, North Carolina sample of March loblolly pine bark were oleic acid and linoleic acids with only a trace of

stearic acid. The earlier August Fraction 0 contained only a little linoleic acid and no oleic acid whatsoever.

Although these two fractions were obtained by different processing schemes, the differences obtained cannot be accounted for on this basis. The original bark extractives must have been different, and the causes may be any of the following: (a) difference in time of year of sampling, (b) difference in location of growth and growing conditions, (c) difference in age of tree, (d) possible disease or other biochemical affliction, and (e) possible mistake in original identification of sample. The problem has not been resolved at this point.

The data of Tables XXIV and XXV demonstrate the occurrence of other fatty acids in the two fractions covered by these two tables. Besides the oleic/linoleic-stearic difference, other acidic components are different. Palmitic acid and undecanoic acid are major components of the March bark extractives after saponification, but they exist only in traces, if at all, in the August bark.

Silica gel chromatography of Fraction L-PE-A. A sample of this acid fraction containing 0.5 g. of solids was absorbed on a little silica gel and packed on top of a dry-packed column of silica gel 18 mm. in diameter and 160 mm. in length. The column was developed with 4:1 chloroform-methanol until the solvent reached the bottom of the column. The column was extruded, and the extruded column was allowed to dry and sectioned into three equal lengths. Each portion was extracted with tetrahydrofuran, and solids analyses on the fractions indicated that 75% of the material applied to the column was in the lower third, 15% in the middle third, and 10% in the upper third. Each fraction was evaporated to dryness, treated with REGISIL, and chromatographed isothermally at 250°C.

under conditions employed in earlier studies. The lower third of the column showed Peaks 7 through 15 of Table XXII with very large peaks for 12 and 15. The middle third showed peaks for 3 through 10, 12 through 15, 19, 23, and 24 with large peaks for 10, 23, and 24. The upper third gave only trace peaks for 15, 19, 23, and 24.

Large-Scale Alkaline Hydrolysis of Petroleum Ether Extractives

A sample of the petroleum ether extractives of the March loblolly pine bark containing 35 g. of solids was evaporated to dryness, and the residue was covered with 500 ml. of 4% sodium hydroxide solution. The mixture was boiled under reflux for 4 hours, allowed to cool, and extracted with ether. A solid remained insoluble in both the aqueous and ether layer. The solution was filtered and washed with ether. All the ether was combined and concentrated to yield the neutral Fraction L-PE-NN containing 9.0 g. or 25.7% of the petroleum ether extractives.

The aqueous solution was acidified with dilute sulfuric acid and extracted with ether. The ether was concentrated to give the acid Fraction L-PE-AA containing 16.4 g. of solids or 46.9% of the petroleum ether extractives. The insoluble solid was suspended in water, acidified with dilute sulfuric acid, and extracted with ether to give a second acid Fraction L-PE-AA-1 containing 4.5 g. of solids corresponding with 12.9% of the petroleum ether extractives.

Gas chromatography of neutral Fraction L-PE-NN. Temperature-programmed and isothermal gas chromatography of this fraction gave results identical with those of the small-scale experiment Fraction L-PE-N in Tables XX and XXI.

Gas chromatography of acid Fractions L-PE-AA and L-PE-AA-1. Both acid fractions were chromatographed directly on the FFAP column with the oven at 100°C. and programmed immediately at the rate of 6° per minute to 250°. The carrier gas flows were 75 ml. per minute. Data for the two fractions are given in Table XXVI.

TABLE XXVI

TEMPERATURE-PROGRAMMED GAS CHROMATOGRAPHY OF ACIDIC FRACTIONS L-PE-AA AND L-PE-AA-1 AS FREE ACIDS

Peak No.	Retention Time, min.	Peak Height, units[a] L-PE-AA	L-PE-AA-1	Identification
1	5.9	1 (6)		
2	8.9	1 (9)	1 (9)	
3	13.1	3 (39)	3 (39)	
4	17.8	38 (676)	3 (53)	Nonanoic acid
5	18.3		3 (55)	
6	21.5	2 (43)	8 (172)	
7	22.0	1 (22)	5 (110)	Myristic acid
8	22.9	2 (46)	3 (69)	
9	24.1	2 (48)		
10	25.5	1 (25)	3 (77)	
11	26.6	25 (665)	7 (186)	Palmitic acid
12	28.0	2 (56)	1 (28)	
13	29.0		1 (29)	
14	30.2		3 (91)	Stearic acid
15	31.4	51 (1,600)	4 (126)	Oleic acid
16	32.9	36 (1,184)	7 (230)	Linoleic acid
17[b]	34.1	1 (34)		Linolenic acid
18	35.2	1 (35)		
19	36.9	1 (36)	6 (222)	Arachidic acid
20	42.4	1 (42)		
21	47.3	1 (47)	11 (520)	
22	54.4		6 (326)	Behenic acid
23	66.2		15 (995)	Tetracosanoic acid
24	92.5		3 (277)	Hexacosanoic acid

[a] The values in parentheses are products of peak height and retention time.

[b] Peaks 17 through 24 are broad peaks.

The data of Table XXVI demonstrate the effect of separation of the precipitated soaps from the alkaline hydrolysis of the petroleum ether extractives of the loblolly pine bark. A substantial fractionation took place by this processing thus illustrating the difference in solubility of sodium soaps of the several long-chain fatty acids in 4% sodium hydroxide solution.

Fraction L-PE-AA-1 containing the precipitated acids was chromatographed isothermally at 265°C. on the FFAP column to compare results with those of Tables XXIV and XXV. These results are shown in Table XXVII.

TABLE XXVII

ISOTHERMAL GAS CHROMATOGRAPHY OF ACIDIC FRACTION L-PE-AA-1
AS FREE ACIDS

Peak No. from Table XXVI	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
14	5.6	1	6	Stearic acid
15	6.1	2	12	Oleic acid
16	6.6	7	46	Linoleic acid
19	8.8	3	26	Arachidic acid
21	10.2	1	10	
22	13.8	6	83	Behenic acid
23	21.8	12	262	Tetracosanoic acid
24	33.8	2	68	Hexacosanoic acid

Further studies on the isolation and identification of components of the products of alkaline hydrolysis under these conditions were held in abeyance pending the development of a standard suitable procedure for alkaline hydrolysis of petroleum ether extractives.

Alkaline Hydrolysis by Standard Procedure of Petroleum Ether Extractives L-PE

Although the last procedure employed for the alkaline hydrolysis in quantity of the petroleum ether extractives, Fraction L-PE, of March loblolly pine bark, gave a preliminary fractionation of the acidic materials, the difficulties inherent in the filtration of the insoluble soaps obviated the use of the procedure for a standard method. Because it was necessary to employ a standard alkaline hydrolysis procedure in a concurrent investigation on the effect of time of year on the extractives contents of southern pine barks (noted in the Introduction and to be reported in Progress Report Two), a search was made for a more suitable procedure. After several different procedures were tried, the following appeared to be satisfactory and was adopted for general use.

A 5.0-g. sample of Fraction L-PE was dissolved in 100 ml. of 4% ethanolic potassium hydroxide, and the solution was boiled under reflux for 2 hours. The clear solution was diluted with 100 ml. of water and evaporated to approximately 50-ml. volume on the rotating vacuum evaporator. The resulting aqueous solution was diluted with water, acidified with dilute sulfuric acid, and extracted with ether. The ether solution was then extracted with 2% aqueous sodium hydroxide. Evaporation of the residual ether left the neutral Fraction L-PE-NNN containing 24.1% of the original Fraction L-PE. The sodium hydroxide solution was acidified with dilute sulfuric acid and extracted with ether. Upon evaporation, this ether yielded the ether-soluble acid Fraction L-PE-AAA containing 72.2% of original Fraction L-PE. On the chance that some materials were insoluble in ether and remained in the water, the raffinate from the last ether extraction was exactly neutralized with dilute sodium hydroxide and evaporated to dryness. The dry residue was warmed with absolute ethanol and filtered,

and the filtrate evaporated to a small volume to give any water-soluble, ether-insoluble components, Fraction L-PE-W.

Gas chromatography of neutral Fraction L-PE-NNN. This neutral fraction was submitted to gas chromatography under conditions employed as standard conditions for the long-term study to be reported in the next progress report. Under these conditions, the fraction was chromatographed directly on the FFAP column with the oven at 130°C. and programmed immediately to 265°C. at the rate of 6° per minute. The helium carrier gas flows were 75 ml. per minute. Data for this fraction are given in Table XXVIII.

Although the product of peak height and retention time gives a rough general idea of the relative concentration of individual components in a mixture, actual values for individual components can be obtained only by quantitative methods. From Disc integrator data for authentic compounds, the analytical chromatograms for Fraction L-PE-NNN indicated 40.4% tetracosanol and 13.0% hexacosanol.

Gas chromatography of ether-soluble acid Fraction L-PE-AAA. As in the case of the neutral fraction, a standard procedure was also evolved for the gas chromatography of the acid fraction. During the development period it was found that these acid fractions gave exceptionally clean chromatograms as trimethylsilyl derivatives, and a large percentage of the peaks could be identified. Therefore, chromatograms on these acid fractions were determined under quantitative conditions, and actual percentage values were determined from the Disc integrator readings rather than employing peak heights for approximating relative concentrations of components. Where peaks are unidentified, quantitative values recorded were calculated assuming integrator units similar to adjacent identified peaks.

TABLE XXVIII

PROGRAMMED GAS CHROMATOGRAPHY OF NEUTRAL FRACTION L-PE-NNN UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification[a]
1	0.9	3	3	
2	1.4	1	1	
3	1.8	1	2	
4	2.7	1	3	
5	3.6	1	4	
6	4.7	5	23	
7	5.0	28	140	
8	5.5	3	16	Nonanol
9	6.0	10	60	
10	6.6	1	7	
11	7.2	3	22	Decanol
12	9.0	2	18	
13	10.6	5	53	
14	11.4	9	103	
15	12.0	1	12	
16	12.7	1	13	Myristyl alcohol
17	13.2	1	13	
18	14.0	2	28	Pentadecanol
19	14.8	1	15	Decanoic acid
20	15.8	1	16	Undecanoic acid
21	18.7	1	19	Tridecanoic acid
22	19.9	29	476	Nonadecanol
23	21.4	2	43	Arichidyl alcohol
24	21.9	1	22	
25	23.4	2	47	Behenyl alcohol
26	23.9	18	431	
27	25.1	3	75	
28	25.9	1	26	Oleic acid
29	27.2	100+	2,720+	Tetracosanol
30	28.8	1	29	
31	31.3	27	846	Hexacosanol
32	33.9		68	

[a] Identifications were made by identity of retention times of authentic compounds under the same standard conditions. Positive identifications can be established only by isolation and subsequent infrared or mass spectroscopy. Thus, these are only tentative identifications.

In accordance with the standard conditions used in the future for continuing samples on a monthly basis, acid Fraction L-PE-AAA was treated with REGISIL, and the trimethylsilylated product was submitted to temperature-programmed gas chromatography on a column of 3% silicone QF-1 on 100-120 mesh GasChrom Q (obtained from Applied Science Laboratories, State College, Penn.) 6 ft. in length in a 1/4-inch stainless steel column. The oven was at 100°C. and programmed immediately upon injection to 240°C. at the rate of 6° per minute. Helium carrier gas rates were 75 ml. per minute. Results for this acid fraction are given in Table XXIX.

TABLE XXIX

PROGRAMMED GAS CHROMATOGRAPHY OF ACID FRACTION L-PE-AAA UNDER STANDARD CONDITIONS

Peak No.	Retention Time, min.	Yield, %[a]	Identification
1	6.4	0.10	Undecanoic acid
2	13.3	0.10	
3	14.7	2.50	Palmitic acid
4	16.7	9.60	Linoleic acid
5	17.7	20.60	Oleic acid[b]
6	19.0	1.90	
7	19.6	3.20	[c]
8	20.3	19.20	Arachidic acid
9	21.6	6.50	[c]
10	23.1	7.70	Behenic acid
11	24.5	3.25	[c]
12	26.1	12.00	Tetracosanoic acid
13	27.7	0.20	Pentacosanoic acid
14	30.3	3.50	Hexacosanoic acid
		90.35	

[a] Based on solids in acid Fraction L-PE-AAA.

[b] This peak contains a trace of stearic acid as determined by chromatography on an FFAP column.

[c] This peak is probably due to a polyunsaturated long-chain acid of chain length greater than 18.

It was during the development period for a standard gas chromatographic procedure to employ on the acid fractions that discrepancies between gas chromatograms of trimethylsilylated acids and those of the free acids became apparent. When the free acid fractions were chromatographed directly on the FFAP column (as in Table XXVI) peaks appeared for acids such as nonanoic acid which did not appear when the acid fraction was analyzed as the trimethylsilylated derivative. Furthermore, depending upon the temperature or temperature-programmed conditions for the free acid chromatogram on FFAP, the retention time obtained varied considerably. It thus appears that unsaturated acids such as oleic or linoleic or similar unsaturated long-chain acids crack to smaller molecules when subjected to the high temperature of the injection port to form a lower molecular weight saturated acid and various size hydrocarbon fractions which would pass over with the solvent. In the case of oleic acid, cracking would produce nonanoic acid and a nine-carbon hydrocarbon. Instant cracking would yield a retention time on the chromatogram corresponding with that of nonanoic acid. If cracking took place on the column at a temperature reached some moments after injection, then the retention time would correspond with an acid of somewhat higher carbon chain length. This same hypothesis would apply to the direct gas chromatography of the neutral fractions during which process long-chain unsaturated alcohols would crack to smaller chain saturated acids and hydrocarbon residues. In fact, experimental chromatograms demonstrated that free neutral fractions chromatographed directly on FFAP gave peaks for fatty alcohols of chain length much lower than those obtained from the trimethylsilylated fractions chromatographed on silicone columns.

The exact nature of this cracking phenomenon will be studied if this project is continued. Investigations comprising instantaneous hydrogenation just prior to gas chromatography as recommended by Beroza and Sarmiento (6) are contemplated.

Gas chromatography of water-soluble, ether-insoluble Fraction L-PE-W. This fraction, which had been evaporated almost to dryness, was dissolved in 2 ml. of tetrahydrofuran and chromatographed directly on the FFAP column under conditions identical with those used as standard conditions for neutral fractions. Results of this chromatogram are given in Table XXX.

TABLE XXX

PROGRAMMED GAS CHROMATOGRAPHY OF WATER-SOLUBLE FRACTION L-PE-W

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
1	4.0	1	4	
2	5.8	17	99	Trimethylene glycol
3	7.7	1	8	
4	9.5	42	294	2,6-di-tert-butyl-p-cresol
5	13.7	1	14	
6	14.8	53	5,300 +	Glycerol

The identifications noted in Table XXX were all made by collection of the fractions corresponding with the large peaks noted and subjecting these collected fractions to infrared absorption spectroscopy. In all cases, the infrared spectra were identical with the identification noted in the table.

Although 2,6-di-tert-butyl-p-cresol was identified positively in Fraction L-PE-W, it is obvious that such a compound was not a component of native loblolly pine bark. Since this compound is used extensively in agricultural sprays, it is possible that the material was present on the bark used in this study as a residue from an insectide or similar spray.

A quantitative chromatogram was run on the glycerol, and it was found that Fraction L-PE-W contained 130 mg. of glycerol corresponding with 2.6% of the petroleum ether extractives, Fraction L-PE.

Investigation of Water-Soluble, Ethyl Acetate-Insoluble Components

The water extractives of Table XV after ethyl acetate extraction contained 3.4% of the original bark solids. Upon standing in the presence of ethyl acetate for a few weeks, this aqueous raffinate deposited a little solid (L-S). The solution was filtered, and the clear filtrate, Fraction L-W, was chromatographed on paper, developed with 8:2:1 ethyl acetate — pyridine — water, and sprayed with aniline hydrogen phthalate reagent with sugars. The paper chromatograms indicated the presence of fructose, glucose, mannose, and arabinose. Indicator spray noted the presence of sugar acids in this fraction.

Gas Chromatography of this Water-Soluble Fraction L-W

A sample of Fraction L-W containing 5.0 g. of solids was evaporated to dryness under reduced pressure, and the residue was extracted with warm absolute ethanol. The ethanol was evaporated to leave 2.18 g. (43.6%) of solids which were dissolved in tetrahydrofuran for gas chromatography. This was Fraction L-W-1.

A sample of Fraction L-W-1 was treated with REGISIL and subjected to temperature-programmed gas chromatography on the silicone SE-30 column with the oven at 100°C. and programmed immediately to 250°C. at the rate of 6° per minute with helium carrier gas flow rates of 75 ml. per minute. A total of 26 peaks was obtained ranging in retention time from 8.5 to 34.0 min. Except for the peak corresponding with glycerol, none of the retention times corresponded with any components found in any other fraction studied so far. It is expected that

the peaks are due to trimethylsilyl derivatives of a wide variety of carbohydrate and carbohydrate-containing components.

Another sample of Fraction L-W was evaporated to dryness, and this time the residue was extracted directly with tetrahydrofuran. In this case, the extract, Fraction L-W-2, contained only 20% of the starting sample. A gas chromatogram indicated the same components as the ethanol extract, but except for two components, the concentrations were much lower. This is more evidence for the conclusion that carbohydrates and carbohydrate derivatives comprise the major portion of Fraction L-W, because their solubility is usually much less in tetrahydrofuran than in ethanol.

Acid Hydrolysis of Water-Soluble Fraction L-W

A sample of Fraction L-W containing 5.0 g. of solids was evaporated slightly to remove any ethyl acetate and diluted with water to make a volume of 100 ml. This aqueous solution was made approximately 1N in sulfuric acid by addition of 3 ml. of concentrated sulfuric acid, and the resulting solution was boiled under reflux for 4 hours. The hydrolysis mixture was cooled and extracted with ether to give Fraction L-W-E containing 3.7% of the solids of Fraction L-W. The residual aqueous solution was filtered to leave 55.4% insoluble solids. The filtrate was passed through a column of DUOLITE-A-6 anion-exchange resin and concentrated to give Fraction L-W-W containing 40.9% of the solids of Fraction L-W.

Chromatography of water-soluble hydrolysis products, Fraction L-W-W. Paper chromatography of the water-soluble components indicated glucose, fructose, galactose, arabinose, and hydroxymethylfurfural as major components and mannose, xylose, and rhamnose as minor components. The identified sugars were determined quantitatively by a modification of the paper chromatographic procedure of Saeman

and coworkers (7). These data are included in Table XXXI along with similar data for the original Fraction L-W.

TABLE XXXI

QUANTITATIVE ANALYSIS OF SUGARS IN FRACTIONS L-W AND L-W-W

Sugar	Yield, % of fraction L-W	L-W-W
Glucose	13.3	27.3
Galactose	1.4	14.1
Mannose		2.8
Arabinose	3.7	20.8
Xylose	0.5	2.5
Rhamnose	0.5	2.8
Fructose	15.6	7.4
Hydroxymethylfurfural	----	22.2
Total	35.0	99.9

The data of Table XXXI demonstrate that the predominant sugars in the water-soluble Fraction L-W are fructose and glucose, and that the other sugars are formed only after acid hydrolysis, being present as oligomeric polysaccharides or glycosides, probably the former. The loss of fructose to form hydroxymethylfurfural upon boiling with N acid is characteristic of this sugar.

Chromatography of ether-soluble hydrolysis products, Fraction L-W-E. Paper chromatography of the ether-soluble hydrolysis products indicated vanillic acid, p-hydroxybenzoic acid, vanillin, and two unidentified acids and an unidentified aldehyde.

This ether-soluble fraction was treated with REGISIL, and the trimethylsilylated components were gas chromatographed on the SE-30 silicone column under temperature-programmed conditions from 100° to 250°C. at the rate of 6° per minute. Results are given in Table XXXII.

Except for Peak 24 of Table XXXII all the major peaks of this chromatogram were identified. The presence of the phenolic acids and aldehydes suggest glycosides or ester combinations with the sugars which were also liberated upon acid hydrolysis.

Inasmuch as Peak 24 represented a major component of the fraction, a series of larger injections was made. The material corresponding with Peak 24 was collected, and solidified upon cooling. An infrared absorption spectrum indicated an aromatic acid, but no evidence of a phenolic group was obtained. This component has not been identified as yet.

Alkaline Hydrolysis of Water-Soluble Fraction L-W

A 5-g. sample of Fraction L-W was hydrolyzed with aqueous 4% sodium hydroxide exactly as described earlier for the petroleum ether extractives of loblolly pine. The resulting alkaline hydrolysis mixture was cooled, acidified with dilute sulfuric acid, and extracted with ether. The ether was evaporated to leave 0.5 g. of ether-soluble alkaline hydrolysis products, Fraction L-W-EA.

Gas chromatography of ether-soluble alkaline hydrolysis products, Fraction L-W-EA. This fraction was treated with REGISIL and chromatographed under conditions identical with those used for the data of Table XXXII. The results obtained are similar to those obtained in Table XXXII and are given in Table XXXIII.

TABLE XXXII

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE FRACTION L-W-E

Peak No.	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
1	1.7	22		Excess REGISIL
2	2.0	12	24	
3	3.4	6	20	
4	4.2	7	29	Lactic acid
5	4.4	5	22	Glycolic acid
6	5.2	100+	520+	Oxalic acid
7	6.0	5	30	
8	6.4	2	13	
9	7.4	2	15	
10	7.7	1	8	
11	8.1	2	17	
12	8.6	8	69	Pyrocatechol
13	9.2	2	18	
14	10.5	6	63	
15	11.0	1	11	
16	11.5	2	23	
17	12.4	20	248	Vanillin
18	12.9	2	26	
19	13.6	1	14	
20	14.8	15	262	p-Hydroxybenzoic acid
21	15.7	1	16	
22	16.4	1	16	
23	17.4	29	505	Vanillic acid
24	18.6	20	373	
25	19.8	2	40	
26	48.5	2	97	
27	52.4	1	52	

TABLE XXXIII

PROGRAMMED GAS CHROMATOGRAPHY OF ETHER-SOLUBLE FRACTION L-W-EA

Peak No.[a]	Retention Time, min.	Peak Height, units	Product of Peak Height and Retention Time	Identification
1(1)	1.8	20		Excess REGISIL
2(2)	2.2	3	7	
3	3.0	1	3	
4(3)	3.5	5	17	
5(4)	4.8	100+	480+	Lactic acid
6(6)	5.5	61	335	Oxalic acid
7(7)	6.1	6	37	
8	6.7	6	40	
9(9)	7.5	1	8	
10(10)	8.1	1	8	
11(11)	8.8	10	88	
12(12)	9.0	11	99	Pyrocatechol
13(13)	9.7	2	19	
14(14)	10.4	3	31	
15(15)	10.9	1	11	
16(16)	11.4	1	11	
17(17)	12.2	7	85	Vanillin
18(18)	12.6	2	25	
19(19)	13.7	3	41	
20(20)	14.5	6	87	p-Hydroxybenzoic acid
21(21)	15.9	1	16	
22(22)	16.3	1	16	
23(23)	16.8	10	168	Vanillic acid
24	17.3	1	17	[b]
25(24)	18.0	8	144	
26(25)	19.4	1	19	
27	20.3	1	20	
28	22.1	6	133	[c]

[a] Values in parentheses are corresponding peaks from Table XXXII.

[b] This is the unidentified aromatic acid of Peak 24 in Table XXXII.

[c] This major component of Fraction L-W-EA did not appear in Fraction L-W-E of Table XXXII.

Gas chromatography of water-soluble alkaline hydrolysis products, Fraction L-W-WA. The aqueous raffinate from the ether extraction of Fraction L-W-WE was passed through a column of AMBERLITE IR-120 cation-exchange resin, and the acid solution was concentrated to a small volume under reduced pressure. The concentrated solution was covered with benzene and dried by boiling under a water-separatory head. After all water was removed, the dry benzene solution was concentrated. A sample was treated with REGISIL and chromatographed exactly as the last two chromatograms. The chromatogram was essentially the same as that of Table XXXII qualitatively, but quantitatively the individual peaks were substantially different. Lactic acid and an unidentified component with a retention time of 6.7 min. (corresponding with Peak 8 of Table XXXIII) were the two major components of this fraction along with unidentified components corresponding with Peaks 16, 18, and 19 of Table XXXII and one with a retention time of 32.0 min. which did not appear in any previous chromatogram.

Investigation of Precipitated Solid from Water Extractives, Fraction L-S

The solid which separated from the water-soluble, ethyl acetate-insoluble components upon standing was hydrolyzed with both acid and with alkali as described for Fraction L-W, and both hydrolyzates were processed as described for the prior fractions. Gas chromatography of the ether extractives of the alkaline hydrolyzate gave results essentially similar to those of Table XXXIII with the major peaks corresponding with Peaks 5, 20, and 25 of Table XXXIII. Similarly, gas chromatography of the ether extractives of acid hydrolysis of Fraction L-S gave results essentially similar to those of Table XXXII with major peaks corresponding with Peaks 20 and 24 of Table XXXII.

Thus, it appears that the component responsible for Peak 24 of Table XXXII and Peak 25 of Table XXXIII is an important moiety in the bark of loblolly pine.

LARGE-SCALE INVESTIGATION OF MARCH SLASH PINE BARK

Further studies on the fractions of the March loblolly pine bark will not be reported at this time so that the results of concurrent studies on slash pine bark can be reported.

On March 11, 1968 we received a large sample of fresh slash pine bark from the Kraft Division of West Virginia Pulp and Paper mill in North Charleston, South Carolina. The freshly cut bark had been shipped from North Charleston on March 1, 1968. It was allowed to airdry and was then reduced to dust in a Wiley mill. The airdry bark containing 11.06% moisture was stored in the cold room in polyethylene bags.

Extractions of Bark Dust

This slash pine bark dust was extracted in a manner identical with that described for the large-scale extraction of March loblolly pine bark dust. Extraction results are given in Table XXXIV.

TABLE XXXIV

EXTRACTIONS OF MARCH SLASH PINE BARK DUST

Solvent	Yield Extractives, %, original bark
Petroleum ether (b.r. 30-60°C.)	2.3
Water	8.0
Ether	2.2
95% Ethanol	0.8
Total	13.3

The hot water extract was then extracted with ethyl acetate as described previously to obtain 20.3% ethyl acetate extractives corresponding with 1.6% of the original bark solids.

Polyamide Chromatography of Ethyl Acetate Extractives

As with the loblolly pine bark extractives, the ethyl acetate extractives were dissolved in tetrahydrofuran, absorbed on polyamide powder, and allowed to airdry. The ethyl acetate extractives absorbed on the polyamide amounted to 37.0 g. The sample mixture was placed on top of a polyamide column of the same dimensions employed in the other experiments and developed with water and ethanol dilutions as follows: Fractions 1 through 90, water; Fractions 91 through 150, 20% ethanol; Fractions 151 through 220, 50% ethanol; Fractions 221 through 250, 95% ethanol; and Fraction 251, hot 95% ethanol extract of the extruded column. A total of 800 ml. of aqueous effluent was discarded before collecting the first 210-ml. fraction in the eluate.

Each fraction was evaporated to dryness on the vacuum rotating evaporator, weighed, and monitored by thin-layer chromatography. The eluate fractions were then grouped and combined on the basis of the monitoring thin-layer chromatograms to give the fractions of Table XXXV.

The total recovery of material from the chromatogram of Table XXXV was only 81%. As in the last experiment, none of these fractions deposited crystals upon concentration.

Gas Chromatography of Combined Fractions

All combined fractions of Table XXXV were dissolved in an appropriate solvent, treated with REGISIL, and subjected to temperature-programmed gas

TABLE XXXV

POLYAMIDE CHROMATOGRAPHY OF ETHYL ACETATE-SOLUBLE PORTION OF HOT WATER EXTRACTIVES OF MARCH SLASH PINE BARK

Fraction	Eluate Fractions	Yield, g.
S-A	1	0.11
S-B	2 - 10	6.33
S-C	11 - 21	2.39
S-D	22 - 27	0.82
S-E	28 - 43	1.79
S-F	44 - 52	0.67
S-G	53 - 76	1.43
S-H	77 - 114	2.67
S-I	115 - 130	1.30
S-J	131 - 162	3.45
S-K	163 - 178	4.01
S-L	179 - 191	0.63
S-M	192 - 212	1.18
S-N	213 - 230	0.91
S-O	231 - 250	1.11
S-P	251	1.31
		30.11

chromatography exactly as detailed for the August loblolly pine bark experiment. Fractions S-A through S-J were dissolved in dimethylformamide, Fractions S-K and S-L in a mixture of dimethylformamide and tetrahydrofuran, and Fractions S-M through S-O in tetrahydrofuran. Fraction S-P was insoluble in any solvent used for this purpose. The results of the temperature-programmed gas chromatography of these slash pine bark fractions are given in Tables XXXVI, XXXVII, and XXXVIII. Again, Fraction S-A was not included in the gas chromatographic investigation because monitoring indicated no components of interest.

TABLE XXXVI

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH SLASH PINE BARK EXTRACTIVES FRACTIONS S-B THROUGH S-F

Peak No.	Retention Time, min.	Peak Height for Fractions, units				
		S-B	S-C	S-D	S-E	S-F
1	3.2	1				
2	3.4	2				
3	4.0	4	1	1	1	2
4	5.8	1				
5	6.3	1				
6	7.8				2	
7	8.1	34	1			
8	8.6	2	1			
9	9.5	1				
10	10.3	1	2	5		
11	10.8				1	1
12	11.1	1				
13	11.5	1	1			
14	12.2	2	12	7	4	4
15	12.9	7	2	5		1
16	13.3			6	2	1
17	13.9	2		1		
18	15.1	7	7	7	13	4
19	15.4	4				
20	15.7		5			
21	16.0		4			
22	16.3				1	1
23	16.5	2				
24	16.9	6	13	1	3	
25	17.2					60

TABLE XXXVI (Continued)

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH SLASH PINE BARK EXTRACTIVES FRACTIONS S-B THROUGH S-F

Peak No.	Retention Time, min.	Peak Height for Fractions, units S-B	S-C	S-D	S-E	S-F
26	17.6	1	2	2	2	
27	18.3	19	12			
28	18.4					11
29	18.7	11	2	1	3	
30	19.1	5	6	2		
31	19.4	4		2		
32	19.5				15	2
33	20.1	2	1	1		
34	20.4	20	1	1	1	
35	20.7					1
36	20.9	3	2			
37	21.7	8				
38	21.8					1
39	22.5	1	2			
40	23.8				1	3
41	24.4				1	
42	24.7				1	
43	24.9	1	1	1	2	6
44[a]	25.6		1			
45	25.8	8	1	1		
46	27.4					1
47	27.9				1	1
48	29.7	1	1			
49	30.2				1	2
50	30.7		1			
51	31.3			2	2	
52	31.5	2				
53	31.6					1
54	33.2		1	1		
55	36.7				1	
56	40.7			1	5	
57	43.0					3
58	43.6		2	1	1	
59	48.4				1	
60	49.4					3

[a]Peaks 44 through 60 are broad peaks.

TABLE XXXVII

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH SLASH PINE BARK EXTRACTIVES FRACTIONS S-G THROUGH S-K

Peak No.	Retention Time, min.	Peak Height for Fractions, units S-G	S-H	S-I	S-J	S-K	Matching Peak in Table XXXVI
1	2.9	3	2	3	2	1	
2	4.3	1	1	1	1	1	3
3	12.8	3	3	1	2	3	14
4	14.2	1	1				
5	15.2	41					
6	17.7	1	1				25
7	18.9	100+	1	1			28
8	19.8	8					32
9	20.3		4	5			
10	20.6	1	2	18	4		35
11	21.0					2	
12	21.1			1			
13	21.2	11					
14	22.0					2	
15	22.1	1	1				38
16	22.7			24	1		
17	23.2					1	
18	23.4	1	100+	84	2	1	40
19	24.2	1					41
20	24.3					1	
21[a]	25.4					2	
22	26.3					1	
23	28.2	2	1				47
24	28.5				3	1	
25	28.7	1					
26	29.3				1	1	
27	30.1	1					49
28	30.9	1	1	1	1		51
29	32.9		1				
30	35.5		1				
31	40.7			46	4	3	
32	42.8			4			
33	44.2		8				
34[b]	45.4			1	23	7	
35	46.8	2	9				
36[c]	46.9				6		
37	49.9		3				
38	51.1		1	4			

[a] Peaks 21 through 38 are broad peaks.
[b] This component was identified as dihydroquercetin.
[c] This component was identified as dihydromyricetin.

TABLE XXXVIII

PROGRAMMED GAS CHROMATOGRAPHY OF MARCH SLASH PINE BARK EXTRACTIVES FRACTIONS S-L THROUGH S-O

Peak No.	Retention Time, min.	Peak Height for Fractions, units S-L	S-M	S-N	S-O	Matching Peak in Table XXXVII
1	3.1	4	1	2		1
2	3.3			1		
3	4.4	2	1	2		2
4	12.4	9	2	2	8	3
5	16.1	1				
6	18.5	2	2	1		
7	21.0	8	3			11
8	22.0	24	4			14
9	23.1	3	4	4	10	16
10	23.7	2	1			18
11	24.1	1	2	4	13	20
12[a]	25.0	14	25	27	53	21
13	40.0		3	3		
14	54.3			1		

[a]Peaks 12 through 14 are broad peaks.

Investigation of Individual Extractives Fractions from March Slash Pine Bark

Every fractionation and analysis applied to March loblolly pine bark extractives was also applied to March slash pine bark extractives, and every loblolly fraction such as L-PE-A or L-W-W had an analogous slash pine Fraction S-PE-A or S-W-W. In almost all instances qualitative results were identical with those obtained with the loblolly pine bark extractives, but quantitative differences in some cases were considerable. In the interest of time and space, the slash pine bark fractions results will not be reported in this progress report. However, the results for all fractions of slash pine bark are on file, and if any individual cooperator is interested in these results, we shall be happy to supply them to him.

In continuing studies on the effect of season on the nature of the components of these two southern pine barks, we found that differences between individual logs of the same species at times are greater than the differences between the two species.

GENERAL DISCUSSION

The experimental results of the present progress report have elucidated a number of important facts. They have demonstrated that the individual components of southern pine bark extractives can be separated so that they can be identified and characterized. A number of major components of loblolly and slash pine bark extractives have been isolated and identified. These studies have also demonstrated that barks of the same species obtained at different times and from distinctly different locations may be different in their chemical component make-up. Thus, the major fatty acid in an August loblolly pine bark from Cedar Springs, Georgia was stearic acid, whereas a March loblolly pine bark from Canton, North Carolina contained almost no stearic acid, but contained oleic and linoleic acids as the major fatty acids.

It was found that best analysis of the total extractives of the southern pine barks could be obtained by sequential extraction of the bark dust with petroleum ether, water, ether, and ethanol. Except for the August loblolly pine bark from Cedar Springs, Georgia, no great differences appeared between loblolly and slash pine barks. In almost all instances, qualitative composition was identical, and differences appeared only in quantitative amounts.

Petroleum ether extractives comprise some free acids, but mostly waxes and some fats. Predominant acids obtained on hydrolysis of these fats and waxes were oleic, linoleic, arichidic, tetracosanoic, and hexacosanoic. Predominant

alcohols obtained under the same conditions were glycerol, octadecanol, tetracosanol, and hexacosanol. The 24-carbon chain acid and alcohol appear to be very important in these waxes.

Water-soluble materials present per se in quantity include dihydromyricetin and dihydroquercetin and the sugars, glucose and fructose. Upon hydrolysis, the water-soluble components yield pyrocatechol, vanillin, vanillic acid, p-hydroxybenzoic acid, oxalic acid, arabinose, galactose, and an unidentified aromatic acid as major components.

The composition of the minor ether and ethanol extractives are still under study and will be reported in the next progress report.

FUTURE STUDIES

In the course of the processing of individual fractions of this progress report a number of unidentified components of importance were found. The identification of some of these are under investigation, and results will be forthcoming in the next progress report.

The next progress report will be concerned chiefly with the effect of month of the year upon the yield and nature of extractive components of loblolly and slash pine barks and upon the yield and nature of the components of the turpentine composition of the loblolly and slash pinewoods. The study will not be complete until the March, 1969 barks and woods are evaluated.

During the comparison study of monthly samples of southern pine barks, a gradual evolution of procedure and knowledge took place in our laboratories. Many of the problems and unknowns present at the initiation of this investigation and noted in this progress report were solved and will be reported in Progress Report Two.

Many others are solved only in part, and many new ones arose during the development period. It is hoped that the project will be continued so that we can complete this fundamental investigation of the components of southern pine barks and include development studies on the isolation and possible production of major identified components.

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